Remarks

Claims 8 - 10, 12, and 24 have been cancelled.
Claims 9, 13, and 25 have been amended.
New claims 28 to 32 have been added.

Independent claims 13, 25, and 28 now include limitations in order to overcome the objections raised.

35 USC 112

To overcome the objection raised to claims 9 and 10, the term "preventing" no longer appears in the claims. Claims 9 and 10 have been cancelled.

35 USC 102

To overcome the objection that certain claims are anticipated by either Williams et al., Berger et al., or Schroten, independent claims 13, 25 and 28 now include limitations not described in these references. These limitations are consistent with limitations put forward in the independent claim proceeding in the counterpart European patent EP 1 613 332 B1 during Opposition Proceedings.

Claim 13 is now limited to the adult dosage of 100 mg to 1 g per day. This is not taught by any of Williams et al, Berger et al., or Schroten. Of these references only Williams et al. and Schroten even addresses dosages and they disclose the use of far lower dosages than required by the present claims to bring about the required change in lipid components for mediating inflammation or reducing plasma cholesterol levels. Thus, there is no disclosure or even a suggestion provided by these references that would lead one of skill in the art to use a sufficient dosage to bring about the required change in lipid components and thereby lower plasma cholesterol or mediate inflammation.

Support for this dosage can be found in the application at paragraph [0251] which states:

> **[0251]** To accomplish an inflammation mediating effect or a cholesterol lowering effect, a typical dosage for adults may be from about 100 mg to about 1 g of ganglioside per person per day, based on an adult body weight of about 70 kg.

Claim 13 now defines "mediating inflammation" as changing lipid components in microdomains for treating inflammatory bowel disorders, disorders arising from allergic responses, diseases involving epithelial surface responses, or inflammation of the intestine, retina, or neuronal tissue. Part of this wording, relating to treating the disorders, was found in previous claim 10. The limitation of changing lipid components in microdomains was described in the application as filed, and is now included in the claims in the interests of further distinguishing the claims from the prior art.

Claim 13 now states that mediating inflammation comprises *changes in lipid components in microdomains*. Further, claim 13 goes on to specify three possible parameters to evaluate changes in lipid components as: (1) a reduction in *platelet activating factor (PAF)*, (2) a reduction in the *ratio of cholesterol:sphingolipid*, or (3) a reduction in *total diglyceride* in the microdomains.

The examples included in the application illustrate these three specific changes in the microdomains, and it is these changes that are associated with the prevention or treatment of inflammatory bowel disorders or diseases involving epithelial surface responses, or mediating inflammation of the intestine, retina, or neuronal tissue. Paragraphs [0010] to [0016] of the application support the rationale behind the microdomain lipid composition being indicative of inflammatory response. For ease of reference, these paragraphs are provided below:

> **[0010]*Microdomains*. Microdomains**, generally called lipid rafts, caveolae, or glycosphingolipid-signaling domains, have been characterized as important domains for signal transduction and lipid (i.e. cholesterol) and protein trafficking (Anderson, 1998; Brown et al., 1998; Hakomori et al., 2000; and Simons et al. 1997). Microdomains are recently known as a site for the cellular entry of bacterial and viral pathogens (Fantini, 2000; Katagiriet al., 1999; and Bavari et al., 2002). For instance, the entry of filoviruses requires lipid rafts as the site of virus attack (Bavari et al., 2002). Cholera toxin entered the cell by endocytosis GM1 as the sorting motif necessary for retrograde trafficking into host cells and

such trafficking depends on association with lipid rafts (Wolf et al., 2002).

[0011] Physiological and functional roles of **microdomains** are dependent on cholesterol and sphingolipids including gangliosides. Reduction of cholesterol inhibits pathogen entry by disrupting the structure of **microdomains** (Popik et al., 2002; Samuel et al., 2001) and impairs inflammatory signalling (Wolf et al., 2002; Triantafilou et al., 2002). Cholesterol upregulates the expression of caveolin, a marker of protein for caveolae (Fielding et al., 1997; Hailstones et al., 1998). Sphingolipid depletion inhibits the intracellular trafficking of GPI-anchored proteins and endocytosis via GPI-anchored proteins (Kasahara et al., 1999), suggesting that lipid-protein interaction directly modulates gene expression and cellular trafficking important for cell development and behavior.

[0012] The neonatal intestine has permeable, endocytic and enzymatic transport systems for absorption of nutrients and immunoglobulins (Moxey et al.,1979; Wilson et al., 1991) but is susceptible to pathogen entry because of higher permeability than that of adults (Koldovsky 1994). High amount of gangliosides in mothers' milk during the neonatal period therefore act as a receptor for viral and bacterial toxins to protect entry of pathogens into enterocytes (Rueda et al., 1998). During development, membrane permeability gradually decreases (Koldovsky 1994) while peptidases and glycosidases become functionally active and enriched in **microdomains** (Danielsen et al., 1995). Many digestive/absorptive enzymes, such as alkaline phosphatase, aminopeptidase N and A, and sucrase-isomaltase are also increased in apical membrane **microdomains** (Stulnig et al., 2001). These results seem to suggest the importance of microdomains of intestinal apical membranes for nutrient uptake and metabolism.

[0013] Polyunsaturated fatty acids (20:5n-3 or 22:6n-3) can accumulate in **microdomains** and displace functional proteins by changing the lipid composition of the **microdomain** (Stulnig et al., 2001; Williams et al., 1999). This observation highlights the importance of dietary lipids in modulating physiological and biological properties of proteins in the microdomain. Little is known of how dietary gangliosides affect the lipid profile and protein components of **microdomains** during neonatal gut development.

[0014] Some previous studies have suggested that cholesterol depletion inhibits inflammatory signaling by disrupting **microdomains** structure (Wolf et al., 2002; Samuel et al., 2001; Triantafilou et al., 2002). However, it has not been evaluated whether diet-induced cholesterol reduction has any effect on decreasing cholesterol in the **microdomain**; disrupting **microdomain** structure and reducing pro-inflammatory mediators such as diglyceride (DG) and platelet activating factor (PAF). DG derived from phospholipids by phospholipase C, binds to protein kinase C (PKC) to phosphorylate targeted proteins, such as the epidermal growth factor receptor and DG resides in **microdomains** (Sciorra et al., 1999; Smart et al., 1995). The instant invention assesses these effects.

[0015] PAF, 1-O-alkyl-2-acetyl-sn-glycero-3-phosphorylcholine, stimulates inflammatory cells such as leukocytes (Prescott et al., 1990) and activates phospholipase A2 (PLA2) in the intestinal tissue to release arachidonic acid (Okayasu et al., 1987). Meanwhile, increased lyso-PC by PLA2 is further used for PAF synthesis with an acetylcholine transferase. PAF binds its receptor to increase intracellular calcium and inositol triphosphate (IP3) production and PKC activation for inflammation (Flickinger et al., 1999). It is unknown if PAF also localizes in the microdomain. Since several studies reported that sphingomyelin (SM), a sphingolipid, has an inhibitory effect on PLA2 activity (Koumanov et al., 1997), it was of interest to determine if dietary ganglioside also decreases PAF synthesis either by increasing sphingolipids or by disrupting **microdomains** structure in developing intestine. We also examine if dietary ganglioside reduces DG content in the **microdomain** since sphingosine, a derivative of sphingolipids inhibits PKC signaling which is required a structural complex with DG.

[0016] Neonates consume SPL including gangliosides from mothers milk (Carlson 1985; Berger et al., 2000). Gangliosides are known to act as receptors for viruses and toxins (Laegreid et al., 1987; Rolsma et al., 1998), activators for T-cells (Ortaldo et al., 1996) and stimulators for Th-1 and Th-2 cytokine-secreting lymphocytes in neonates (Vazquez et al., 2001). Gangliosides are also one of the major lipid components in **microdomains**. It is not known if dietary ganglioside changes the lipid profile and structure of the intestinal **microdomain** and modulating inflammatory signalling mechanisms in the developing intestine. Thus the objective of the present study was to determine if dietary ganglioside increases gangliosides and decreases cholesterol and caveolin content in the intestinal **microdomain** leading to disruption of **microdomain** structure and anti-inflammatory signals in the developing gut.

Support for the wording relating to each of the three lipid component parameters now found in claim 13 can be found within the patent application as follows:

A Reduction in Platelet Activating Factor (PAF)

Platelet Activating Factor, or PAF is a lipid component found in microdomains also known as 1-O-alkyl-2-acetyl-sn-glycero-3-phosphorylcholine, that stimulates an inflammatory response. Please refer to paragraph [0015] of the patent application.

Figure 6 and **Figure 16** illustrate PAF content of intestinal microdomains after feeding different diets for 2 wks, showing that PAF content is reduced, versus the control diet.

In **Example 4** of the patent, it is emphasized in paragraph [0298] that:

> It is concluded that dietary GG (gangliosides) decrease the cholesterol/GG ratio, caveolin, **PAF** and DG content in microdomains and may have a potential anti-inflammatory effect during gut development.

In **Example 7** of the patent, it is emphasized in paragraph [0401] that:

> The increase in GD3 composition of microdomains for animals fed the ganglioside-enriched diet correlated to a decrease in both **PAF** and DG in microdomains. These parameters are indicative of a decrease in inflammatory factors in the intestine and thus show inflammation mediation induced by dietary gangliosides.

A Reduction in the Ratio of Cholesterol:Sphingolipid

Figure 7 shows the ratio cholesterol/SM (sphingomyelin), (part B) in intestinal microdomains after feeding different diets for 2 wks. The animals fed the ganglioside diet had a reduced ratio versus control animals.

In **Example 4**, a ganglioside-supplemented diet-induced decrease in the ratio of cholesterol to sphingolipids is observed to attenuates the caveolin and inflammatory mediator content in microdomains of the rat intestine.

At paragraph [0320] of the patent application, it is stated:

> These observations suggest that dietary ganglioside increased SM content more than GG content in microdomains since the ratio of cholesterol to SM was more dramatically reduced by 56% in microdomains while the ratio of cholesterol to GG was decreased by 40% when compared to control animals.

A Reduction in Total Diglyceride

Diglyceride is considered a pro-inflammatory mediator. For background, please refer to paragraphs [0014] and [0015].

In **Figure 16**, the composition of diglyceride in microdomains of rat intestine is shown, and it is clear that diglycerides (DG) are reduced with a ganglioside supplemented (GG) diet. In **Example 4**, at paragraph [0324], diglyceride content of microdomains is assessed. Diglyceride is considered an inflammatory mediator:

> Animals fed the GG diet significantly decreased 1,2-DG and total DG by 44% and 43% of controls, respectively....Taken together, these results

> suggest that dietary ganglioside and PUFA have potential anti-inflammatory effects in developing animals and that dietary ganglioside is more effective in reducing two inflammatory factors than feeding PUFA.

Claim 25 finds similar support in the application as put forward above with respect to the amendments made to claim 13, although claim 25 is not limited to a specific dose, and is restricted to reducing plasma cholesterol.

Claim 28 finds support in the rationale put forward in respect of claims 13 and 25. Claim 28 is parallel to claim 13 as amended, except that the dosage recited is pertinent to infants. The passages of support found for claim 13 also apply to claim 28, with the exception of the support for the infant dosage of 10-50 mg/day. This limitation finds support in the patent application at paragraph [0252] which states:

> **[0252]** For an infant having a typical body weight of about 3.5 kg, a level of gangliosides that may be delivered in order to accomplish an inflammation mediating effect may range from about 10 to about 50 mg per day per infant.

The additional limitations now included in independent claims 13, 25 and 28 traverse the objection raised on the basis of Williams et al., Berger et al., or Schroten. None of these documents describe the dosage ranges now indicated, the changing of lipid components in microdomains, nor do these documents refer to a reduction in platelet activating factor (PAF), a reduction in the ratio of cholesterol:sphingolipid, or a reduction in total diglyceride in the microdomains. On this basis, the applied references cannot be considered as anticipatory.

35 USC 103

The applicants also request the examiner to reconsider the rejection for obviousness in view of Williams et al. or Berger and withdraw the rejection. Applicants submit that the new claims put forward herewith cannot be considered obvious in view of those references because they simply do not disclose all of the limitations, such as the requirement to change lipid components in microdomains in order to mediate inflammation or reducing plasma cholesterol levels or the use of sufficient amounts of the ganglioside compositions to accomplish those ends, as are now required by the claims.

Double Patenting

In view of the amendments applicants also kindly request reconsideration and withdrawl of the double patenting and provisional double patenting rejections because the present claims, which now require dosages and/or the requirement to change lipid components in microdomains, contain limitations not found in claims 1-5 of U.S. Patent No. 6,998,392 or in the copending application 11/622858.

In the event the examiner identifies any issues that can be expeditiously resolved by a telephone call, the examiner is encouraged to contact the undersigned.

Respectfully submitted,

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